SEQUENCE LISTING

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The Government of the United States of America
as represented by the Secretary of the
Department of Health and Human Services

<120> Methods for Identifying Inhibitors of GADD45
Polypeptide Activity, and Inhibitors of Such Activity

<130> 015280-367100US

<140> US 09/534,811
<141> 2000-03-24

<150> US 60/126,069
<151> 1999-03-25

<160> 32

<170> PatentIn Ver. 2.1

<210> 1
<211> 1343
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (284)..(781)
<223> human growth arrest and DNA-damage-inducible
protein (GADD45)

<400> 1
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cggggagcga gcgagcaagc aaggcgggag gggtggccgg agctgcggcg gctggcacag 120

gaggaggagc ccgggcgggc gaggggcggc cggagagcgc cagggcctga gctgccggag 180

cggcgcctgt gagtgagtgc agaaagcagg cgcccgcgcg ctagccgtgg caggagcagc 240

ccgcacgccg cgctctctcc ctgggcgacc tgcagtttgc aat atg act ttg gag 295
Met Thr Leu Glu
1

gaa ttc tcg gct gga gag cag aag acc gaa agg atg gat aag gtg ggg 343
Glu Phe Ser Ala Gly Glu Gln Lys Thr Glu Arg Met Asp Lys Val Gly
5 10 15 20

gat gcc ctg gag gaa gtg ctc agc aaa gcc ctg agt cag cgc acg atc 391
Asp Ala Leu Glu Glu Val Leu Ser Lys Ala Leu Ser Gln Arg Thr Ile
25 30 35

act gtc ggg gtg tac gaa gcg gcc aag ctg ctc aac gtc gac ccc gat 439
Thr Val Gly Val Tyr Glu Ala Ala Lys Leu Leu Asn Val Asp Pro Asp
40 45 50

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aac gtg gtg ttg tgc ctg ctg gcg gcg gac gag gac gac gac aga gat   487
Asn Val Val Leu Cys Leu Leu Ala Ala Asp Glu Asp Asp Asp Arg Asp
         55                  60                  65

gtg gct ctg cag atc cac ttc acc ctg atc cag gcg ttt tgc tgc gag   535
Val Ala Leu Gln Ile His Phe Thr Leu Ile Gln Ala Phe Cys Cys Glu
     70                  75                  80

aac gac atc aac atc ctg cgc gtc agc aac ccg ggc cgg ctg gcg gag   583
Asn Asp Ile Asn Ile Leu Arg Val Ser Asn Pro Gly Arg Leu Ala Glu
 85                  90                  95                 100

ctc ctg ctc ttg gag acc gac gct ggc ccc gcg gcg agc gag ggc gcc   631
Leu Leu Leu Leu Glu Thr Asp Ala Gly Pro Ala Ala Ser Glu Gly Ala
                105                 110                 115

gag cag ccc ccg gac ctg cac tgc gtg ctg gtg acg aat cca cat tca   679
Glu Gln Pro Pro Asp Leu His Cys Val Leu Val Thr Asn Pro His Ser
            120                 125                 130

tct caa tgg aag gat cct gcc tta agt caa ctt att tgt ttt tgc cgg   727
Ser Gln Trp Lys Asp Pro Ala Leu Ser Gln Leu Ile Cys Phe Cys Arg
        135                 140                 145

gaa agt cgc tac atg gat caa tgg gtt cca gtg att aat ctc cct gaa   775
Glu Ser Arg Tyr Met Asp Gln Trp Val Pro Val Ile Asn Leu Pro Glu
    150                 155                 160

cgg tga tggcatctga atgaaaataa ctgaaccaaa ttgcactgaa gtttttgaaa    831
Arg
165

tacctttgta gttactcaag cagttactcc ctacactgat gcaaggatta cagaaactga 891

tgccaagggg ctgagtgagt tcaactacat gttctggggg cccggagata gatgactttg 951

cagatggaaa gaggtgaaaa tgaagaagga agctgtgttg aaacagaaaa ataagtcaaa 1011

aggaacaaaa attacaaaga accatgcagg aaggaaaact atgtattaat ttagaatggt 1071

tgagttacat taaaataaac caaatatgtt aaagtttaag tgtgcagcca tagtttgggt 1131

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accatatttt attgtatttt gatgagatat taaattctca aagttttatt ataaattcta 1251

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<210> 2
<211> 165
<212> PRT
<213> Homo sapiens

<400> 2

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Met Thr Leu Glu Glu Phe Ser Ala Gly Glu Gln Lys Thr Glu Arg Met
  1               5                  10                  15
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Asp Lys Val Gly Asp Ala Leu Glu Glu Val Leu Ser Lys Ala Leu Ser
             20                  25                  30

Gln Arg Thr Ile Thr Val Gly Val Tyr Glu Ala Ala Lys Leu Leu Asn
         35                  40                  45

Val Asp Pro Asp Asn Val Val Leu Cys Leu Leu Ala Ala Asp Glu Asp
     50                  55                  60

Asp Asp Arg Asp Val Ala Leu Gln Ile His Phe Thr Leu Ile Gln Ala
 65                  70                  75                  80

Phe Cys Cys Glu Asn Asp Ile Asn Ile Leu Arg Val Ser Asn Pro Gly
                 85                  90                  95

Arg Leu Ala Glu Leu Leu Leu Leu Glu Thr Asp Ala Gly Pro Ala Ala
            100                 105                 110

Ser Glu Gly Ala Glu Gln Pro Pro Asp Leu His Cys Val Leu Val Thr
        115                 120                 125

Asn Pro His Ser Ser Gln Trp Lys Asp Pro Ala Leu Ser Gln Leu Ile
    130                 135                 140

Cys Phe Cys Arg Glu Ser Arg Tyr Met Asp Gln Trp Val Pro Val Ile
145                 150                 155                 160

Asn Leu Pro Glu Arg
                165
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<210> 3
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:PCR
amplification primer

<400> 3
ggcggctcga gactttggag gaattctcgg c 31

<210> 4
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:PCR
amplification primer

<400> 4
catcaccgtt cagggagatt aatc 24

<210> 5
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:GADD45
subsequence acidic motif amino acid residues
62-67; exemplary peptide inhibiting GADD45-related
dissociation of Cdc2/cyclin B1 complexes

<400> 5
Asp Glu Asp Asp Asp Arg
1 5

<210> 6
<211> 160
<212> PRT
<213> Homo sapiens

<220>
<223> human growth arrest and DNA-damage-inducible
protein (hGADD45beta)

<400> 6
Met Thr Leu Glu Glu Leu Val Ala Cys Asp Asn Ala Ala Gln Lys Met
1 5 10 15

Gln Thr Val Thr Ala Ala Val Glu Glu Leu Leu Val Ala Ala Gln Arg
20 25 30

Gln Asp Arg Leu Thr Val Gly Val Tyr Glu Ser Ala Lys Leu Met Asn
35 40 45

Val Asp Pro Asp Ser Val Val Leu Cys Leu Leu Ala Ile Asp Glu Glu
50 55 60

Glu Glu Asp Asp Ile Ala Leu Gln Ile His Phe Thr Leu Ile Gln Ser
65 70 75 80

Phe Cys Cys Asp Asn Asp Ile Asn Ile Val Arg Val Ser Gly Met Gln
85 90 95

Arg Leu Ala Gln Leu Leu Gly Glu Pro Ala Glu Thr Gln Gly Thr Thr
100 105 110

Glu Ala Arg Asp Leu His Cys Leu Leu Val Thr Asn Pro His Thr Asp
115 120 125

Ala Trp Lys Ser His Gly Leu Val Glu Val Ala Ser Tyr Cys Glu Glu
130 135 140

Ser Arg Gly Asn Asn Gln Trp Val Pro Tyr Ile Ser Leu Gln Glu Arg
145 150 155 160

<210> 7
<211> 159
<212> PRT
<213> Homo sapiens

<220>
<223> human growth arrest and DNA-damage-inducible protein (hGADD45gamma)

<400> 7
Met Thr Leu Glu Glu Val Arg Gly Gln Asp Thr Val Pro Glu Ser Thr
1 5 10 15

Ala Arg Met Gln Gly Ala Gly Lys Ala Leu His Glu Leu Leu Leu Ser
20 25 30

Ala Gln Arg Gln Gly Cys Leu Thr Ala Gly Val Tyr Glu Ser Ala Lys
35 40 45

Val Leu Asn Val Asp Pro Asp Asn Val Thr Phe Cys Val Leu Ala Ala
50 55 60

Gly Glu Glu Asp Glu Gly Asp Ile Ala Leu Gln Ile His Phe Thr Leu
65 70 75 80

Ile Gln Ala Phe Cys Cys Glu Asn Asp Ile Asp Ile Val Arg Val Gly
85 90 95

Asp Val Gln Arg Leu Ala Ala Ile Val Gly Ala Gly Glu Glu Ala Gly
100 105 110

Ala Pro Gly Asp Leu His Cys Ile Leu Ile Ser Asn Pro Asn Glu Asp
115 120 125

Ala Trp Lys Asp Pro Ala Leu Glu Lys Leu Ser Leu Phe Cys Glu Glu
130 135 140

Ser Arg Ser Val Asn Asp Trp Val Pro Ser Ile Thr Leu Pro Glu
145 150 155

<210> 8
<211> 165
<212> PRT
<213> Mus musculus

<220>
<223> mouse growth arrest and DNA-damage-inducible protein (mGADD45)

<400> 8
Met Thr Leu Glu Glu Phe Ser Ala Ala Glu Gln Lys Thr Glu Arg Met
1 5 10 15

Asp Thr Val Gly Asp Ala Leu Glu Glu Val Leu Ser Lys Ala Arg Ser
20 25 30

Gln Arg Thr Ile Thr Val Gly Val Tyr Glu Ala Ala Lys Leu Leu Asn
35 40 45

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Val Asp Pro Asp Asn Val Val Leu Cys Leu Leu Ala Ala Asp Glu Asp
     50                  55                  60

Asp Asp Arg Asp Val Ala Leu Gln Ile His Phe Thr Leu Ile Arg Ala
 65                  70                  75                  80

Phe Cys Cys Glu Asn Asp Ile Asn Ile Leu Arg Val Ser Asn Pro Gly
                85                  90                  95

Arg Leu Ala Glu Leu Leu Leu Leu Glu Asn Asp Ala Gly Pro Ala Glu
            100                 105                 110

Ser Gly Gly Ala Ala Gln Thr Pro Asp Leu His Cys Val Leu Val Thr
        115                 120                 125

Asn Pro His Ser Ser Gln Trp Lys Asp Pro Ala Leu Ser Gln Leu Ile
    130                 135                 140

Cys Phe Cys Arg Glu Ser Arg Tyr Met Asp Gln Trp Val Pro Val Ile
145                 150                 155                 160

Asn Leu Pro Glu Arg
                165
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<210> 9
<211> 165
<212> PRT
<213> Rattus norvegicus

<220>
<223> rat growth arrest and DNA-damage-inducible protein
(rGADD45)

<400> 9

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  1                 5                  10                  15

Asp Thr Val Gly Asp Ala Leu Glu Glu Val Leu Ser Lys Ala Arg Ser
             20                  25                  30

Gln Arg Thr Ile Thr Val Gly Val Tyr Glu Ala Ala Lys Leu Leu Asn
         35                  40                  45

Val Asp Pro Asp Asn Val Val Leu Cys Leu Leu Ala Ala Asp Glu Asp
     50                  55                  60

Asp Asp Arg Asp Val Ala Leu Gln Ile His Phe Thr Leu Ile Arg Ala
 65                  70                  75                  80

Phe Cys Cys Glu Asn Asp Ile Asn Ile Leu Arg Val Ser Asn Pro Gly
                85                  90                  95

Arg Leu Ala Glu Leu Leu Leu Leu Glu Asn Asp Lys Ser Pro Ala Glu
            100                 105                 110

Ser Gly Gly Leu Ala Gln Thr Pro Asp Leu His Cys Val Leu Val Thr
        115                 120                 125

Asn Pro His Ser Ser Gln Trp Lys Asp Pro Ala Leu Ser Gln Leu Ile
    130                 135                 140
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Cys Phe Cys Arg Glu Ser Arg Tyr Met Asp Gln Trp Val Pro Val Ile
145 150 155 160

Asn Leu Pro Glu Arg
165

<210> 10
<211> 34
<212> PRT
<213> Homo sapiens

<220>
<223> human GADD45 residues 58-91

<400> 10
Leu Leu Ala Ala Asp Glu Asp Asp Asp Arg Asp Val Ala Leu Gln Ile
1 5 10 15

His Phe Thr Leu Ile Gln Ala Phe Cys Cys Glu Asn Asp Ile Asn Ile
20 25 30

Leu Arg

<210> 11
<211> 34
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:human GADD45
residues 58-91 with residues 62-67 changed to Ala
by site-directed mutagenesis (M62-67)

<400> 11
Leu Leu Ala Ala Ala Ala Ala Ala Ala Ala Asp Val Ala Leu Gln Ile
1 5 10 15

His Phe Thr Leu Ile Gln Ala Phe Cys Cys Glu Asn Asp Ile Asn Ile
20 25 30

Leu Arg

<210> 12
<211> 34
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:human GADD45
residues 58-91 with residues 74-79 changed to Ala
by site-directed mutagenesis (M74-79)

<400> 12
Leu Leu Ala Ala Asp Glu Asp Asp Asp Arg Asp Val Ala Leu Gln Ile
1 5 10 15

Ala Ala Ala Ala Ala Ala Ala Phe Cys Cys Glu Asn Asp Ile Asn Ile
20 25 30

Leu Arg

<210> 13
<211> 34
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:human GADD45
residues 58-91 with residues 82-87 changed to Ala
by site-directed mutagenesis (M82-87)

<400> 13
Leu Leu Ala Ala Asp Glu Asp Asp Asp Arg Asp Val Ala Leu Gln Ile
1 5 10 15

His Phe Thr Leu Ile Gln Ala Phe Ala Ala Ala Ala Ala Ala Asn Ile
20 25 30

Leu Arg

<210> 14
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Ran small
nuclear GTPase carboxy-terminal domain closely
related acidic motif

<400> 14
Asp Glu Asp Asp Asp Leu
1 5

<210> 15
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:GADD45 acidic
motif residues 62-69

<400> 15
Asp Glu Asp Asp Asp Arg Asp
1 5

<210> 16
<211> 46
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:exemplary peptide inhibiting GADD45-related dissociation of Cdc2/cyclin B1 complexes

<400> 16
Glu Ala Ala Lys Leu Leu Asn Val Asp Pro Asp Asn Val Val Leu Cys
1 5 10 15

Leu Leu Ala Ala Asp Glu Asp Asp Asp Arg Asp Val Ala Leu Gln Ile
20 25 30

His Phe Thr Leu Ile Gln Ala Phe Cys Cys Glu Asn Asp Ile
35 40 45

<210> 17
<211> 38
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:exemplary peptide inhibiting GADD45-related dissociation of Cdc2/cyclin B1 complexes

<400> 17
Leu Leu Asn Val Asp Pro Asp Asn Val Val Leu Cys Leu Leu Ala Ala
1 5 10 15

Asp Glu Asp Asp Asp Arg Asp Val Ala Leu Gln Ile His Phe Thr Leu
20 25 30

Ile Gln Ala Phe Cys Cys
35

<210> 18
<211> 26
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:exemplary peptide inhibiting GADD45-related dissociation of Cdc2/cyclin B1 complexes

<400> 18
Asp Asn Val Val Leu Cys Leu Leu Ala Ala Asp Glu Asp Asp Asp Arg
1 5 10 15

Asp Val Ala Leu Gln Ile His Phe Thr Leu
20 25

<210> 19
<211> 21
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:exemplary peptide inhibiting GADD45-related dissociation of Cdc2/cyclin B1 complexes

<400> 19
Cys Leu Leu Ala Ala Asp Glu Asp Asp Asp Arg Asp Val Ala Leu Gln
1 5 10 15

Ile His Phe Thr Leu
20

<210> 20
<211> 21
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:exemplary peptide inhibiting GADD45-related dissociation of Cdc2/cyclin B1 complexes

<400> 20
Asp Asn Val Val Leu Cys Leu Leu Ala Ala Asp Glu Asp Asp Asp Arg
1 5 10 15

Asp Val Ala Leu Gln
20

<210> 21
<211> 26
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:exemplary peptide inhibiting GADD45-related dissociation of Cdc2/cyclin B1 complexes

<400> 21
Glu Ala Ala Lys Leu Leu Asn Val Asp Pro Asp Asn Val Val Leu Cys
1 5 10 15

Leu Leu Ala Ala Asp Glu Asp Asp Asp Arg
20 25

<210> 22
<211> 26
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:exemplary peptide inhibiting GADD45-related dissociation of Cdc2/cyclin B1 complexes

<400> 22
Asp Glu Asp Asp Asp Arg Asp Val Ala Leu Gln Ile His Phe Thr Leu
1 5 10 15

Ile Gln Ala Phe Cys Cys Glu Asn Asp Ile
20 25

<210> 23
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:exemplary peptide inhibiting GADD45-related dissociation of Cdc2/cyclin B1 complexes

<400> 23
Ala Asp Glu Asp Asp Asp Arg Asp Val Ala Leu Gln Ile His Phe Thr
1 5 10 15

Leu Ile Gln Ala Phe Cys Cys Glu Asn Asp Ile
20 25

<210> 24
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:exemplary peptide inhibiting GADD45-related dissociation of Cdc2/cyclin B1 complexes

<400> 24
Ala Asp Glu Asp Asp Asp Arg Asp Val Ala Leu Gln Ile His Phe Thr
1 5 10 15

Leu

<210> 25
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:exemplary peptide inhibiting GADD45-related dissociation of Cdc2/cyclin B1 complexes

<400> 25
Ala Asp Glu Asp Asp Asp Arg Asp Val Ala Leu Gln
1 5 10

<210> 26
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:exemplary peptide inhibiting GADD45-related dissociation of Cdc2/cyclin B1 complexes

<400> 26
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1 5

<210> 27
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:exemplary peptide inhibiting GADD45-related dissociation of Cdc2/cyclin B1 complexes

<400> 27
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1 5 10 15

<210> 28
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:exemplary peptide inhibiting GADD45-related dissociation of Cdc2/cyclin B1 complexes

<400> 28
Cys Leu Leu Ala Ala Asp Glu Asp Asp Asp Arg
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<210> 29
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:exemplary peptide inhibiting GADD45-related dissociation of Cdc2/cyclin B1 complexes

<400> 29
Cys Leu Leu Ala Ala Asp Glu Asp Asp Asp Arg Asp
1 5 10

<210> 30
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:exemplary peptide inhibiting GADD45-related dissociation of Cdc2/cyclin B1 complexes

<400> 30
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1 5 10 15

<210> 31
<211> 36
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:exemplary peptide inhibiting GADD45-related dissociation of Cdc2/cyclin B1 complexes

<400> 31
Asp Asn Val Val Leu Cys Leu Leu Ala Ala Asp Glu Asp Asp Asp Arg
1 5 10 15

Asp Val Ala Leu Gln Ile His Phe Thr Leu Ile Gln Ala Phe Cys Cys
20 25 30

Glu Asn Asp Ile
35

<210> 32
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:exemplary peptide inhibiting GADD45-related dissociation of Cdc2/cyclin B1 complexes

<400> 32
Leu Asn Val Asp Pro Asp Asn Val Val Leu Cys Leu Leu Ala Ala Asp
1 5 10 15

Glu Asp Asp Asp Arg Asp Val Ala Leu Gln Ile His Phe Thr Leu Ile
20 25 30

Gln Ala Phe Cys Cys Glu Asn Asp Ile
35 40